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TECHNICAL ADVANCE

Arabidopsis microarrays identify conserved and differentially expressed genes involved in shoot growth and development from distantly related plant species

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Summary

Expressed sequence tags (EST)-based microarrays are powerful tools for gene discovery and signal transduction studies in a small number of well-characterized species. To explore the usefulness of this technique for poorly characterized species, we have hybridized the 11 522-element *Arabidopsis* microarrays with labeled cDNAs from mature leaf and shoot apices from several different species. Expression of 23 to 47% of the genes on the array was detected, demonstrating that a large number of genes from distantly related species can be surveyed on *Arabidopsis* arrays. Differential expression of genes with known functions was indicative of the physiological state of the tissues tested. Genes involved in cell division, stress responses, and development were conserved and expressed preferentially in growing shoots.

Keywords: microarray, development, heterologous hybridization, growth.

Introduction

Arabidopsis thaliana has been used as a model plant system for studying all aspects of plant development and physiological responses (Meyerowitz *et al.*, 1991), and considerable effort has been directed at developing molecular tools for this species (The Arabidopsis Genome Initiative, 2000). For example, the entire *Arabidopsis* genome is sequenced, and tagged mutagenized seed stocks, extensive EST and gene expression databases, and commercially produced cDNA microarrays are available. Such efforts have led to an enviable state of knowledge and tools for directed studies of plant physiology and development, unattainable by those researching other less-characterized plant systems. It is not known how, or if, these *Arabidopsis*-specific tools may be used to study less characterized plant systems. However, conserved ortholog marker sets for dicotyledonous plants have been identified (Fulton *et al.*, 2002), suggesting that hybridization-based tools, such as the microarrays, offer the greatest possibility for the study of heterologous systems. Until now, use of microarrays has been limited to a small subset of well-characterized plant species, such as *Arabidopsis*, maize, and rice (Girke *et al.*, 2000; Kawasaki *et al.*, 2001; McGonigle *et al.*, 2000). The small number of suitable organisms places a limit on the biological processes that can be studied.

None of the well-characterized model plants possess vegetative buds or maintain a perennial life cycle. Thus, global expression of genes associated with regulating growth of vegetative buds has not been studied in detail. Understanding the signals that control growth and development of shoot apices is of prime concern to those attempting to develop ways to control weeds, increase crop production, and decrease crop losses because of inappropriate germination of seeds and tubers.

We set out to determine if cDNA microarrays from *Arabidopsis* can be used to study the processes of growth and development in poorly characterized plant systems. We

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compared the gene expression in mature leaves, which are rich in non-growing or slowly growing cells, to the gene expression in growing shoot apices, which are rich in dividing cells. These two tissue types are common to most plant species and allow comparisons of gene expression patterns for identification of common signaling pathways and conserved genes. We performed analogous assays using similar material from wild oat (*Avena fatua*), an important annual weedy grass species; poplar (*Populus deltoidsies*), a tree species with agronomic importance; leafy spurge (*Euphorbia esula*), an invasive perennial weed; and *A. thaliana*. The results suggest that *Arabidopsis* microarrays can be used to study the transcriptomes of these diverse species.

Results

Hybridization of heterologous RNA from several plant species

Arabidopsis cDNA microarrays were used to study the transcriptomes from leaf and shoot apices of four plant species: *Arabidopsis*, leafy spurge, poplar, and wild oat. At least two biological replicates of samples for each of these species were hybridized to the arrays. An additional technical replicate for each of the two sample sets from *Arabidopsis* was also analyzed, and two technical replicates of a third biological replicate were analyzed for leafy spurge.

Over 47% of the 11 522 clones present on the Arabidopsis Functional Genomics Consortium microarrays consistently produced a signal greater than threshold levels (four times that of average non-*Arabidopsis* controls) when hybridized to leafy spurge cDNAs (see Supplementary data 3). Over 34% of the *Arabidopsis* clones produced signals that were greater than threshold levels when hybridized to poplar and 23% when hybridized to wild oat. These values represent a minimum percentage of hybridizing clones. When labeled *Arabidopsis* cDNAs from similar tissues were hybridized to identical arrays, 70% of the clones consistently hybridized above threshold levels (see Supplementary data 4 for description of threshold level). A representative section of the arrays shows the similarity in hybridization to individual clones (Figure 1).

Only 10% of the genes present on the array hybridized to *Arabidopsis*, but did not hybridize to any of the heterologous labeled cDNAs (see Supplementary data 3). Likewise, 3% of the genes on the array were expressed at greater than four times the background levels only in leafy spurge, while 0.3% of the genes were uniquely expressed in poplar, and 0.03% in wild oat (see Supplementary data 3). Genes represented by clones TIGR loci Atg15090, Atg17380, and EST 150J1T7 (does not correspond to any TIGR open reading frame locus) were consistently expressed at greater than 13 times over background in leafy spurge, wild oat, and poplar, but were not expressed above four times over background in *Arabidopsis*. The gene represented by Atg15090 encodes a putative zinc-binding dehydrogenase. Genes represented by ESTs Atg17380 and 150J1T7 have no known function. Fifteen genes were expressed only in the annual species (*Arabidopsis* and wild oat), and 59 different genes only in the perennial species (see Supplementary data 3).

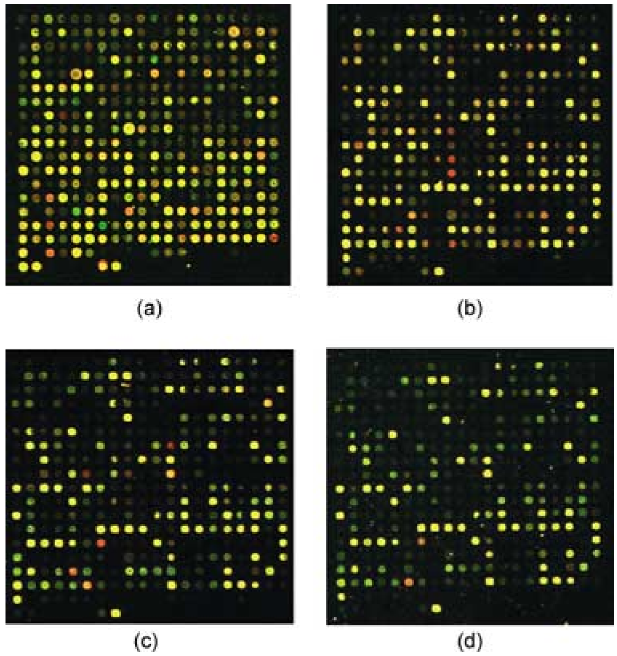

Figure 1. Representative section of *Arabidopsis* array (grid one of AFGC series 2001) hybridized with labeled cDNA derived from leaf and shoot apices RNA from (a) *Arabidopsis*, (b) leafy spurge, (c) poplar, and (d) wild oat.

Differential hybridization to heterologous cDNAs from leaves and shoot apices

Between 0.2 and 2.0% of the hybridizing genes were statistically differentially expressed at least twofold. A full list of the differentially expressed genes and average hybridization ratios is presented in Supplementary data 1. Analyses of the hybridizations indicate that 150 genes were identified as being differentially expressed when the transcriptomes of leaves were compared to those of shoot apices in *Arabidopsis*. Likewise, 56 differentially expressed genes were identified in leafy spurge, 39 in poplar, and 14 in wild oat.

Cluster analysis indicated that many of the genes that were consistently expressed preferentially in the leaves of more than one species were recognizably involved in photosynthesis. Likewise, many of the genes expressed preferen-

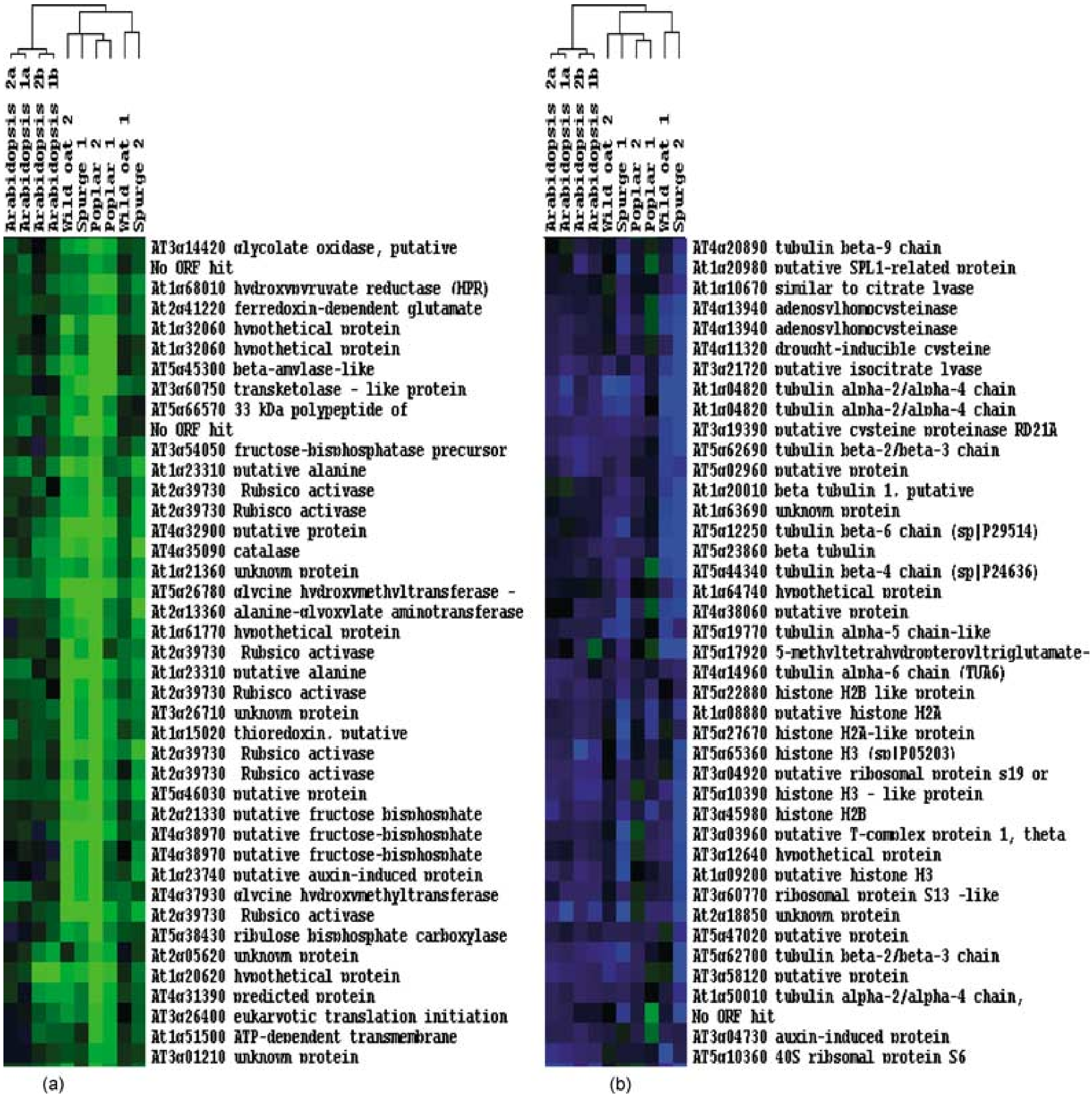

Figure 2. Cluster analysis of (a) genes expressed preferentially in the mature leaves and (b) those expressed preferentially in the growing shoot apices. Color intensity is directly relative to magnitude of differential expression ratios between leaf and shoot apices for the denoted genes (right) in each replicate of the experiment (top). Expression ratios were transformed to log base 2 and subjected to average linkage clustering. Publicly available clustering and tree viewing programs were used to develop and present the results.

tially in the shoot apices were involved in cell division and growth (Figure 2). Two hundred and thirty-five genes were differentially expressed in at least one species, and 23 were differentially expressed in at least two species (Table 1).

Two of the 12 genes preferentially expressed in the shoot apices of more than one species were histones. Three others were tubulin genes, and two others encoded 60S ribosomal protein. The remainder included a gene that encoded putative signaling proteins, a gene involved in DNA synthesis, and three genes with unknown function. One of the genes with unknown function, At1g63690, has a structure indicative of cytokine receptors.

Northern analysis of putative differentially expressed genes

Homologs of seven genes identified as differentially expressed by heterologous microarray analysis were avail-

Table 1 Genes that hybridized preferentially to labeled cDNAs derived from mature leaves or shoot apices from the designated species

		Relative fold induction in			
Locus	Function	*Arabidopsis*	Spurge	Wild oat	Poplar
Up in shoot					
At1g26910	60S RIBOSOMAL PROTEIN L10	5.1	3.3		
At1g70600	60S RIBOSOMAL PROTEIN L27A	5.0	3.0		
At5g10390	HISTONE H3	5.5	7.8		
At5g65360	Histone H3 (H3-1.1)	4.8	4.7		
At5g10520	Pto kinase interactor-like protein		2.5	2.6	
At2g21790	Ribonucleoside-diphosphate reductase large subunit	6.2	2.6		
At1g04820	Tubulin alpha-2/alpha-4 chain		6.8	7.6	
At1g20010	TUBULIN BETA-5 CHAIN′		4.8	2.9	
At5g23860	TUBULIN BETA-8 CHAIN		7.0	4.9	
At2g18850	Unknown protein	5.9	7.7		
At1g63690	Unknown protein		4.0	3.7	
At3g58460	Unknown protein		11.8		3.8
Up in leaves					
At2g39730	Ribulose bisphosphate carboxylase/oxygenase activase		3.3	2.6	4.6
At3g26710	Unknown protein			2.6	4.8
At4g32900	Putative protein		3.1	2.8	6.9
At4g37930	Glycine hydroxymethyltransferase-like protein			4.9	5.1
At4g38970	Plastidic aldolase		3.3	2.9	6.4
At5g26780	Glycine hydroxymethyltransferase-like protein			3.3	5.9
At5g45170	Putative protein			2.9	3.8
At5g60680	Unknown protein		3.0		8.5
At1g61770	Unknown protein		3.3		3.8
At2g13360	Alanine:glyoxylate aminotransferase		3.7		3.1

able from a small collection of sequenced cDNAs of leafy spurge. Northern analysis confirmed the differential expression of these in leafy spurge (Figure 3). Several *Arabidopsis* cDNAs were obtained and used directly to probe these blots. The *Arabidopsis* cDNAs also hybridized to differentially expressed RNAs in leafy spurge (Figure 3). Other genes from our collection were present on the arrays and were shown to be differentially expressed by Northern analysis, but were not differentially expressed above threshold levels in both microarray experiments. However, with the exception of an ortholog of At3g56940 (*At103*), all were differentially expressed at greater than 1.5-fold on the arrays.

Cluster analysis of gene expression in Arabidopsis *for genes expressed preferentially in the apices of leafy spurge*

Our primary interest in beginning this project was to study vegetative reproduction and control of growth in leafy spurge. We used cluster analysis to study the expression pattern of the 44 genes preferentially expressed in shoots of leafy spurge across all the experiments available in the Stanford Microarray Database (see Supplementary data 2). Several recognizable clusters were identified (Figure 4). Differentially expressed genes in these clusters were classified based on their probable cellular function. Clusters containing a predominance of histone and tubulin genes flanked a central cluster. The central cluster contained two subclusters dominated by ribosomal genes and an additional set of tubulin and amino acid biosynthesis genes. Genes involved in similar cellular processes were also preferentially expressed in shoot apices of *Arabidopsis*, poplar, and wild oat (see Supplementary data 1).

Differentially expressed genes responsive to growth-regulating signals in leafy spurge

Northern analysis shows that a spurge homolog of *Histone H3* and a gene hybridizing with the *Arabidopsis* clone for *5-methyltetrahydropteroyltriglutamate-homo-cysteine S-methyltransferase* (At5g17920) are directly responsive to conditions that influence cell division (Figure 5). These genes are upregulated in buds, following treatments known to induce S-phase of cell cycle resulting from treatment with GA_3 or defoliation of the aerial portion of the plant (Horvath *et al.*, 2002). As expected for growth-regulated genes, cold and drought stress result in reduced expression of both these genes. Leafy spurge homologs of *Tubulin* and *Adenosylhomocysteinase* hybridize to RNAs that are also upregulated by treatments that initiate cell division in underground buds, but are not inhibited by cold stress in growing shoot apices. In fact, *Adenosylhomocysteinase* consistently showed greater expression in cold-treated shoot apices than in untreated shoot apices. The *Arabidopsis* clone representing gene At1g63690 showed variable

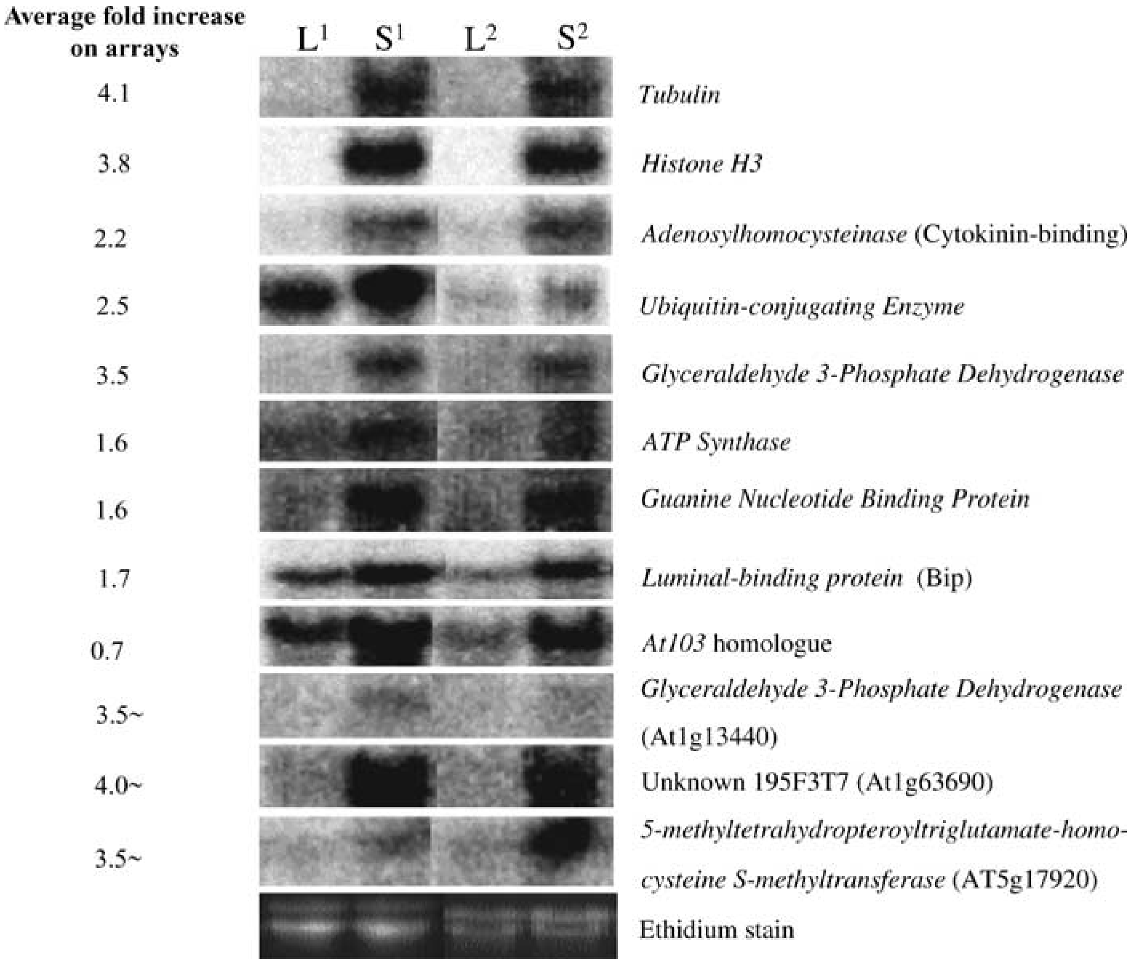

Figure 3. Northern blot analysis of two independently isolated sets of RNA collected from leaf (L^1 and L^2) and shoot apices (S^1 and S^2) hybridized to the designated clones.
Average fold increases observed on microarrays are shown on left. Most probes were produced from a collection of leafy spurge cDNAs with homologs on the *Arabidopsis* microarray. Probes produced directly from *Arabidopsis* cDNA clones are denoted by a '∼'.

induction in underground buds (induced 3 days following defoliation in three of five experiments; partial data shown). At1g63690 was consistently expressed in meristems under both control and cold treatments, but was inhibited following drought stress, like the other genes studied.

Discussion

We have shown that information gained from hybridizing *Arabidopsis* arrays with heterologous cDNAs can identify differentially expressed genes, and may be used directly to characterize transcriptomes in other plant species. We have demonstrated that such genes can provide information on physiological processes, such as vegetative reproduction, that are not usually associated with *Arabidopsis* (see Supplementary data 3). Expression analysis available from extensive *Arabidopsis* microarray databases can provide additional information on signaling pathways for physiological processes that are active in other plants. Finally, because what is learned from hybridizing *Arabidopsis* arrays with heterologous cDNAs is, by design, relevant to multiple species including *Arabidopsis*, such experiments greatly increase the possibilities for functional genomic analysis of conserved physiological processes as well as processes unique to poorly characterized species, such as vegetative growth and bud dormancy.

Heterologous hybridization to microarrays provides an indication of physiological state

Many of the conserved and differentially expressed genes were characteristic of the known physiological processes in the tissues tested. Both histones and tubulins are known to play a role in cell division, and it is not surprising that genes encoding these proteins are both conserved and preferentially expressed in the growing shoot apices (Fobert *et al*., 1994; Vantard *et al*., 2000). Likewise, several genes encoding proteins expressed preferentially in the leaves play an obvious role in photosynthesis and sugar production/

Figure 4. Tree diagram from cluster analysis for 43 genes preferentially expressed in shoot apices of leafy spurge on the AFGC arrays.
Clustering was based on expression of these 43 genes in 575 different experiments available in the Stanford Microarray Database. Functional categories for each TIGR locus were those derived automatically from the PEDANT database (http://pedant.gsf.de/). Gene function for each TIGR locus was obtained from the MIPS database (http://mips.gsf.de/proj/thal/db/search/search_frame.html). Colored bars indicate various subclusters (light gray = flanking clusters, dark gray-stripped = central cluster).

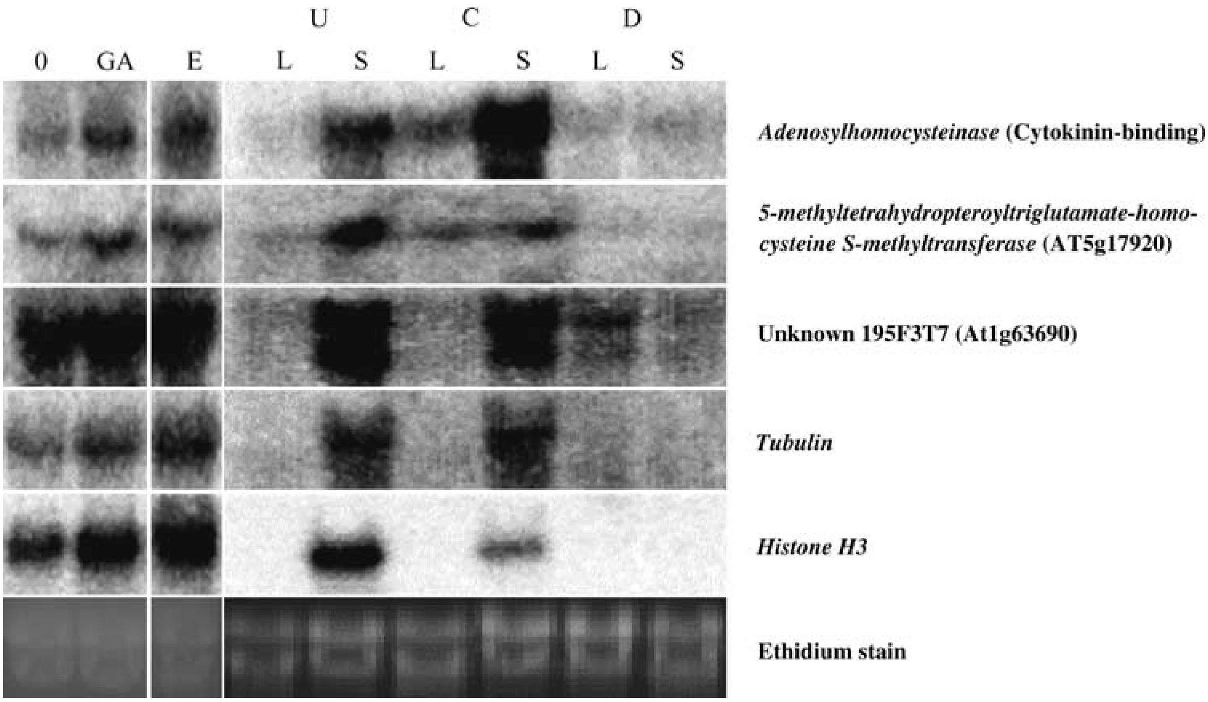

Figure 5. Northern blot of RNA collected from underground buds of leafy spurge, untreated (0), or 3 days following treatment with gibberellic acid (GA), or excision (E) of the aerial portion of the plant.
Also, RNA was collected from untreated (U), cold-stressed (C), or drought-stressed (D) tissue from leaves (L) or shoot apices (S). Blots were sequentially hybridized to the designated probes. All hybridizations for each probe were done in the same reaction on two separate filters (one with samples from underground buds after defoliation and hormone treatments and one from shoot apices and leaves after stress). Blots showing effects of stress and effects of hormone and defoliation were sequentially hybridized with the designated probes.

transport. Such proteins include Rubisco activase, thioredoxin, glycine hydroxymethyltransferase, catalase, and transketolase. However, most of the genes that were expressed preferentially in the leaves of two or more species were not differentially expressed in *Arabidopsis*. This suggests that very young and expanding leaves of *Arabidopsis* are fully functional in contrast to the young expanding leaves of the other species tested.

Heterologous hybridization is consistent with orthologous nature of genes

The physiological relevance of these experiments indicates that many heterologous cDNAs hybridize to their orthologs in *Arabidopsis*. Additional analysis indicates that the substantial level of hybridization correlates better with the presence of orthologous than with random sequence similarity. Screening a leafy spurge cDNA library using *Arabidopsis* cDNA probes identified nine different genes. These genes had varying degrees of hybridization efficiency on the *Arabidopsis* array ranging from four times to greater than 40 times over background (data not shown). Sequence analysis of all resulting clones indicates that they share a homology to their *Arabidopsis* counterparts that is greater at the amino acid level than at the nucleic acid level. Also, 25 randomly chosen unigene sequences, obtained from our leafy spurge EST database, were screened for homology to *Arabidopsis* genes by BLASTN and BLASTX searches against the GenBank database. Approximately, 60% of the leafy spurge EST sequences had over 78% sequence similarity to greater than 100 bp stretches from known *Arabidopsis* genes. One hundred percent of the sampled sequences had higher identity at the amino acid sequence level than at the nucleic acid level. Together, these results indicate that there is sufficient homology within the expressed sequences of these diverse plants to allow meaningful heterologous hybridization of microarrays.

With homologous hybridization to microarrays, samples may not be specific enough to distinguish between individual genes within gene families, but increasing stringency of the hybridization can result in greater specificity without appreciable loss of signal. This is unlikely to be the case with heterologous hybridization. Also, it may prove impractical to increase stringency conditions to limit hybridization to well-conserved domains when using heterologous samples. Although some level of spurious hybridization may occur when using a heterologous sample, the results from these studies demonstrate that sufficient homology exists to gain some insight into specific physiological processes.

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Cluster analysis of differentially expressed genes provides insight into gene function

Cluster analysis provided information on the possible function of some genes of unknown function (see Supplementary data 2). Two genes of unknown function, represented by At2g18850 and At3g58460, cluster near a histone-encoding gene, suggesting that they may play a role in S-phase of the cell cycle. One of these, At2g18850, has substantial homology to a known histone-lysine *N*-methyltransferase-encoding gene. One other gene with unknown function, represented by At1g63690, clusters tightly with the tubulin-encoding genes. Detailed structural and expression analysis of the At1g63690 clone suggests that it might play a role in cytoskeletal alterations in response to cold stress, cell division, and development (unpublished results).

Cluster analysis indicates similarities between Arabidopsis *and leafy spurge shoot development*

Cluster analysis identified the signal transduction pathways common between leafy spurge and *Arabidopsis*. Many of the histone-, tubulin-, and ribosomal protein-encoding genes appear to be responsive to growth-stimulating treatments in *Arabidopsis*. These genes are preferentially expressed in growing tissues, such as dark grown callus, independent of the developmental state of the tissue (see Supplementary data 2). Several of these same genes were induced in growing tissues of leafy spurge (Horvath and Anderson, 2002; Horvath *et al*., 2002). However, a subset of the tubulin-encoding genes and the gene represented by At1g63690 appear to require, or respond to, developmental signals as well in both *Arabidopsis* and leafy spurge (see Supplementary data 2) (Horvath and Anderson, 2002; Horvath *et al*., 2002). Excluding the histone subcluster, a majority of the genes in the central cluster are responsive to developmental programs that induce meristem formation, such as overexpression of *Kn1*. These genes are also responsive to conditions or treatments that alter the levels of the plant hormones abscisic acid (ABA) and gibberellic acid (GA), such as the *abi1* mutation and GA treatment in both *Arabidopsis* and leafy spurge (see Supplementary data 2) (Horvath and Anderson, 2002; Horvath *et al*., 2002). Consequently, these gene clusters may be useful in studying the separation and cross-talk of signaling pathways regulating cell division, and those responding to developmental and hormonal signals.

Evolutionary implications of heterologous microarray results

We have gleaned information on evolutionary trends from our data by requiring stringent conditions for designation of a successful hybridization (see Supplementary data 3 for groupings of expressed genes by species and reproduction methods and cycles). The results provide a potential mechanism for studying plant evolution that goes beyond simple sequence comparisons. The evolution and conservation of specific signaling pathways should provide a better understanding of how and why various species have evolved to meet the requirements of their particular environment. Similar studies have already been attempted to identify signaling pathways that play a role in ecotype specificity and evolution in yeast (Ferea *et al*., 1999).

We identified several groups of genes that were only expressed or differentially expressed in the tissues tested for a particular species. *Adenosylhomocysteinase* (At4g-13940) and *Phosphoethanolamine N-methyltransferase* (At1g48600) are expressed preferentially in shoot apices of leafy spurge, but not in the shoots of the other plants tested. These genes have been implicated in alterations of plant morphology and are likely to be involved in methylation of DNA. Altered expression of *Adenosylhomocysteinase* has been shown to induce changes in plant morphology (Tanaka *et al*., 1997).

Approximately 33% of the genes uniquely expressed in leafy spurge for this study with a putative function have potential regulatory roles. Notable among them are *Argonaute, Brassinosteroid insensitive 1*, several protein kinases, and transcription factors. Differing levels of *Brassinosteroid insensitive 1* and *Argonaute* might be expected when comparing rosette plants to non-rosette plants. Surprisingly, no pattern of gene expression illuminated any fundamental differences between species with different life cycles.

Experimental procedures

Plant material

Leafy spurge: Plant material used for these experiments consisted of mature leaves or tight shoot whorls with outer leaves removed from more than 20 different 3-month-old greenhouse-grown plants cultured from shoot cuttings as a small group of plants that were originally isolated from a wild *E. esula* L. population in North Dakota. Shoot cuttings were placed in Sunshine mix and grown in $1' \times 8'$ cones in a greenhouse under an 18 h photoperiod at 28 ± 4°C for 3 months. Plants used for all the studies described in this paper were single stems.

Poplar: Mature leaves or shoot terminals from lateral branches were collected in early and late summer from three wild trees grown in Fargo, ND.

Wild oat: Mature leaves or emerging tillers were collected from more than 15 greenhouse-grown plants.

Arabidopsis: The largest four leaves or the meristem including the two smallest distinguishable leaves were collected from more than 100 2-week-old greenhouse-grown plants. Two separate sets of leaf and shoot apices were collected at different times and from different plants for each species to serve as biological replicates. All harvested tissues from individual replicates were separately pooled and frozen in liquid N_2 and stored at $-80^\circ C$ until RNA was extracted.

To test the effects of drought and cold stress on gene expression in leaves and shoot apices of leafy spurge, plants were placed in a growth chamber with a constant temperature of $5^\circ C$ under an 18 h photoperiod. Mature leaves as well as shoot apices were harvested separately from 21 individual plants 5–6 days following initiation of cold stress. Alternatively, water was withheld from the plants until plants were wilted (5–7 days). Relative water content was measured at 78 and 64%, respectively, for two separate experiments. Again, mature leaves as well as shoot apices were harvested from 21 individual plants for RNA extraction.

To test the effects of GA_3 and defoliation on gene expression in underground buds, plants were watered once with 25 ml of a 0.5% Tween 20 solution with or without 1 mM GA_3 (potassium salt), or the entire aerial portion of the plant was excised. All distinguishable underground buds 0.25 mm or larger were harvested 3 days following treatment.

Microarray design, gene identification procedures, database searches, and microarray analysis procedures

Microarray analysis was done using methods developed for the *Arabidopsis* Functional Genomics Consortium (Schaffer *et al*., 2001). These arrays contain 11 522 cDNA clones representing approximately 7500 *Arabidopsis* individual gene family members and single-copy genes, as well as 14 non-plant cDNAs spotted in duplicate.

Biological sample sets (total or mRNA from leaf and shoot apices) from each species were labeled and reverse labeled with Cy3 and Cy5 dyes, respectively, and hybridized to an array. Additional technical replicates were performed on each of the two *Arabidopsis* sample sets for a total of 10 separate hybridizations for these experiments. Genes were considered 'expressed' if the hybridization intensity was fourfold or greater than the average hybridization intensity for the bacterial and mammalian control cDNAs in one or both channels. Genes were considered differentially expressed if the difference in normalized hybridization intensities between cDNAs derived from leaf and shoot apices was greater than twofold after subtracting the standard error on all spots representing a given gene. Loess normalization of the data sets was done according to Yang *et al*. (2002).

Cluster analysis was accomplished using data from the Stanford Microarray Database. Expression values for each of the genes were grouped by experiment. Reciprocal experiments were modified, so the expression was noted as treatment divided by control. Expression values for similar experiments were averaged. Clusters were developed by the software and procedures detailed by Eisen *et al*. (1998) using default parameters.

Iowa State University's DNA Sequencing Facility performed sequence analysis of leafy spurge cDNAs. Identification of heterologous genes was accomplished by BLASTP searches using the predicted amino acid sequence from leafy spurge clones. Cluster analysis was done using the clustering and tree-viewing programs developed and made public by Eisen *et al*. (1998).

Northern blot analysis

RNA was collected using the Pine Tree Extraction method (Chang *et al*., 1993). Total RNA (50 μg) was separated on denaturing agarose gels and blotted according to standard techniques (Sambrook *et al*., 1989). Radiolabeled probes for the genes were prepared and hybridized to the various blots (5× SCC/50% formamide) at $42^\circ C$ overnight. Blots were washed four times in 2× SSC, 0.2% SDS at room temperature for 5 min each, and then two times at $65^\circ C$ for 15 min each. The resulting hybridizations were visualized on a Packard Instant Imager®. Linearity was maintained for all of the images presented. Northern blot analyses were carried out with at least two independent sets of RNA. All probes used for Northern analysis were either genes from leafy spurge that had been identified from an existing sequenced EST set or *Arabidopsis* ESTs that were shown to successfully identify, by hybridization and subsequent sequence similarity, leafy spurge cDNAs. Genetic material used for generating probes for these studies was obtained from a leafy spurge EST database developed from a cDNA library made using underground buds harvested 3 days after defoliation.

Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/TPJ/1706/TPJ1706sm.htm

Supplementary data 1 A list of genes with average differential expression values greater than 2 minus the standard error is presented for each species studied. The average expression ratios for each gene along with values of standard error are presented for each species.

Supplementary data 2 Cluster analysis of all *Arabidopsis* microarrays posted to the Stanford Microarray Database. Clusters represent expression patterns of genes preferentially expressed in growing meristems of leafy spurge on *Arabidopsis* arrays. Expression ratios for the genes were modified to accurately represent control/treated so that reciprocal experiments could be clustered together and averaged.

Supplementary data 3 A list of genes expressed in both biological replicates, at levels greater than fourfold of the average hybridization intensity of non-*Arabidopsis* controls, on the AFGC arrays are presented. Clone ID and the organ in which the gene is expressed are shown for each species. Also listed are the genes expressed only in a given species. Additional comparisons list genes expressed only in annual, perennial, and vegetatively reproducing species. A list of genes lacking consistent hybridization in any tissue for all species is shown.

Supplementary data 4 Values for the minimum intensity level of genes considered to be expressed. Values calculated are based on the parameters described. Also shown are the number and the percentage of genes with expression levels greater than fourfold over non-*Arabidopsis* controls on the AFGC arrays.

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